INTERNATIONAL STANDARD

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Surface active agents — Determination of chloroacetic acid (chloroacetate) in surfactants —

Part 1: HPLC method

Agents de surface — Détermination de l'acide chloroacétique (chloroacétate) dans les agents tensioactifs —

Partie 1: Méthode CLHP

Reference number
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Foreword

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The committee responsible for this document is ISO/TC 91, *Surface active agents*.

ISO 17293 consists of the following parts, under the general title *Surface active agents — Determination of chloroacetic acid (chloroacetate) in surfactants*:

— *Part 1: HPLC method*

— *Part 2: Ionic chromatographic method*

Surface active agents — Determination of chloroacetic acid (chloroacetate) in surfactants —

Part 1: HPLC method

1 Scope

This part of ISO 17293 specifies a method for the determination of monochloroacetic acid (monochloroacetate) and dichloroacetic acid (dichloroacetate) in surfactants by HPLC method.

The method applies for anionic surfactants such as alkyl (phenyl) ethoxylated carboxylate (AEC) or amphoteric surfactants such as alkyl imidazoline carboxylate, alkyl dimethyl betaine, and fatty acetyl propyl dimethyl betaine.

The limit of detection (LOD) is ≤0,3 µg/ml for monochloroacetic acid and ≤0,2 µg/ml for dichloroacetic acid; the limit of quantification (LOQ) is ≤1,0 µg/ml for monochloroacetic acid and ≤0,75 µg/ml for dichloroacetic acid (using a standard solution).

The LOD, at 5 g of sample weight, is ≤6 mg/kg for monochloroacetic acid and ≤4 mg/kg for dichloroacetic acid; and the LOQ is ≤20 mg/kg for monochloroacetic acid and ≤15 mg/kg for dichloroacetic acid.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 607, *Surface active agents and detergents — Methods of sample division*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

3 Principle

The sample is dissolved in the mobile phase in order to analyse by high performance liquid chromatography (HPLC). After injection, it flows through a C_8-bonded silicone gel column. The monochloroacetic acid (monochloroacetate) and dichloroacetic acid (dichloroacetate) are separated in the column and detected by an UV detector.

The contents of monochloroacetic acid and dichloroacetic acid in the sample are achieved by external calibration method.

4 Reagents

4.1 General

During the analysis, use only reagents of recognized analytical grade and the water used shall conform to grade 1 in accordance with ISO 3696, unless otherwise specified.

4.2 **Monochloroacetic acid ($ClCH_2COOH$)**, purity > 99 % (w/w).

4.3 **Dichloroacetic acid ($Cl_2CHCOOH$)**, purity > 99 % (w/w).

4.4 **Acetonitrile (CH_3CN) HPLC grade**, filtered before use with filter unit (5.7).

4.5 **Phosphoric acid (H_3PO_4)**.

4.6 **Hydrochloric acid (HCl)**.

4.7 **Hydrochloric acid solution**, 1:1 (V/V).

Add to about 10 ml of hydrochloric acid (4.6) and 10 ml of water in portions. Mix well.

5 Apparatus

Use usual laboratory apparatus and, in particular, the following.

5.1 **HPLC instrument**, equipped with pump and a high-resolution UV detector or photodiode array detector, with the noise and the drift of baseline at 254 nm < 2×10^{-5} AU/s (blank cell) and at 254 nm < 1×10^{-3} AU/h (blank cell, after stabilizing for 60 min), respectively.

5.2 **HPLC column: C_8-bonded phase silicone gel (particle size 5 µm)**, 250 mm × 4,6 mm (ID), pH range from 1 to 8, or equivalent.

5.3 **Filter syringe**, of capacity 2 ml or 5 ml.

5.4 **Injection syringe**, of capacity 25 µl.

5.5 **Analytical balance**, accurate to 0,1 mg.

5.6 **Ultrasonic device**, for the degassing of reagents.

5.7 **Filters with suitable porosity (0,2 µm or 0,45 µm)**, for the filtration of reagents and sample.

5.8 **Vacuum pump**.

5.9 **pH meter**, for pH measurement.

5.10 **Volumetric flasks**, of capacity 50 ml and 100 ml.

5.11 **Glass beakers**, of capacity 50 ml and 100 ml.

6 Procedures

6.1 HPLC conditions

The choice of HPLC conditions depends on the apparatus in use and can be varied from those given below, provided that suitable separation of the compounds of interest is maintained. The following conditions have been found to be suitable for the HPLC column recommended in 5.2.

6.1.1 Mobile phase: Add 100 ml of acetonitrile (4.4) in 900 ml of water, then pipette 2,0 ml of phosphoric acid (4.5) and mix well. Before using, the mobile phase should be degassed with an ultrasonic device (5.6).

6.1.2 Flow rate: 1,0 ml/min.

6.1.3 Detecting wavelength: 214 nm.

6.1.4 Column temperature: room temperature.

6.1.5 Injection volume: 20 µl.

Based on the above conditions, a typical chromatogram is shown in Figure B.1 in Annex B.

6.2 Calibration

a) Weigh 0,1 g of monochloroacetic acid (4.2) and 0,1 g of dichloroacetic acid (4.3), accurate to 0,1 mg, in a 50 ml beaker, add approximately 20 ml of mobile phase (6.1.1) to it, and then stir to thoroughly dissolve. Transfer quantitatively to a 100 ml volumetric flask, make up to the mark with mobile phase, and completely homogenize.

   Store this solution in a refrigerator at (4 ± 2) °C and renew it every 7 d.

   Quantitatively dilute 0,1ml, 0,5 ml, 1,0 ml, 1,5 ml, and 2,5 ml of this solution to 100 ml with mobile phase (6.1.1), respectively. These standards shall be freshly prepared before analysis.

b) Filter (5.7) the diluted solutions and inject 20 µl to the HLPC for analysis. The analysis shall be done at least twice in accordance with the chromatographic conditions given in 6.1. The obtained linear correlation coefficient (R) shall be 0,999 or above.

6.3 Sampling and analysis

Prepare and store the test sample in accordance with ISO 607.

Add approximately 5 g of test sample, accurate to 0,1 mg, in 30 ml of mobile phase (6.1.1), and stir until the sample is dissolved completely. Measure the pH with a pH meter (5.9). Adjust the pH to the same as that of the mobile phase with HCl solution (4.7). Quantitatively dilute the sample to 100 ml with mobile phase (6.1.1). Filter (5.7) it and inject 20 µl of the diluted solution for analysis on the chromatographic conditions given in 6.1.

NOTE If interference to the peak of monochloroacetic acid or dichloroacetic acid in the betaine chromatogram was found during the test of the betaine sample, prepare the sample solution in accordance with the method in Annex A, using a cation-exchange column to remove the interference. After preliminary treatment, this solution shall be filtered (5.7) and injected 20 µl for analysis on the chromatographic conditions given in 6.1.

7 Results and calculation

7.1 General

Determine the monochloroacetic acid and dichloroacetic acid contents in the test sample using the procedure according to 6.3. Calculate the results in milligrams per kilogram (mg/kg) according to Formula (1) and Formula (2).

7.2 The content of monochloroacetic acid

The content of monochloroacetic acid in the test sample is calculated using Formula (1):

$$X_1(\text{mg/kg}) = \frac{A \times V}{m} \quad (1)$$

where

X_1 is the content of monochloroacetic acid, in mg/kg;

A is the calculated concentration of monochloroacetic acid in the test sample solution, in µg/ml;

V is the volume of the test sample, in ml;

m is the mass of the test sample, in g.

7.3 The content of dichloroacetic acid

The content of dichloroacetic acid in the test sample is calculated using Formula (2):

$$X_2(\text{mg/kg}) = \frac{B \times V}{m} \quad (2)$$

where

X_2 is the content of dichloroacetic acid, in mg/kg;

B is the calculated concentration of dichloroacetic acid in the test sample solution, in µg/ml.

8 Precision

8.1 Repeatability limit

The absolute difference between two independent single test results, obtained using the same method on identical test materials in the same laboratory by the same operator using the same equipment within a short interval of time, shall not exceed the repeatability limit (r) in more than 5 % of cases.

According to ISO 5725-2, r can be expected to be

— 10 % for contents greater than or equal to 50 mg/kg, and

— 15 % for contents less than 50 mg/kg.

8.2 Reproducibility limit

The absolute difference between two single test results, obtained using the same method on identical test materials in different laboratories by different operators using different equipment, shall not exceed the reproducibility limit (R) in more than 5 % of cases. R can be expected to be 40 %.

9 Test report

The test report shall contain at least the following information:

a) the test method used, with reference to this part of ISO 17293 (i.e. ISO 17293-1);

b) all information necessary for the complete identification of the test compound;

c) all the data (e.g. in tabular form) obtained and the calibration curve;

d) all operating details not specified in this part of ISO 17293, or regarded as optional, together with details of any incidents which might have influenced the test result(s).

Annex A
(informative)

Preliminary treatment of betaine sample

A.1 Reagents

During the analysis, use only reagents of recognized analytical grade, and the water used shall conform to grade 3 in accordance with ISO 3696, unless otherwise specified.

A.1.1 Cation exchanger (KAT), strongly acidic, 50 to 100 mesh.

A.1.2 Hydrochloric acid, 4 mol/l solution.

Add 700 ml of hydrochloric acid to 1 400 ml of water; mix well.

A.1.3 Acetonitrile (CH_3CN): HPLC grade.

A.1.4 Acetonitrile solution, 10% (V/V).

Add 30 ml of acetonitrile (A.1.3) to 270 ml of water; mix well.

A.2 Apparatus

A.2.1 Volumetric flask, of capacity 100 ml.

A.2.2 Glass beakers, of capacity 200 ml and 3 000 ml.

A.2.3 Glass exchange column with tap, for resin preparation: inner tube 50 mm to 60 mm in diameter and 550 mm to 600 mm in height.

A.2.4 Glass exchange column with tap, for sample analysis: inner tube 15 mm in diameter and 400 mm in height.

A.3 Procedure

A.3.1 Preparation of cation-exchange resin

Place 600 ml of cation-exchange resin (A.1.1) in a 3 000 ml beaker (A.2.2) and cover by adding 2 000 ml of hydrochloric acid (A.1.2). Allow to stand for at least 2 h, with occasional stirring. Decant the acid and transfer the resin into the preparation column (A.2.3) by means of deionized water.

The column should contain a glass-wool plug. Wash the column with water until the eluate is free of chloride.

A.3.2 Cation-exchange column

Place 60 ml of cation-exchange resin in the analytical column (A.2.4) with a glass-wool plug in it. Wash with water until the pH of the eluate reaches 7, approximately. Displace the water with 100 ml of acetonitrile solution (A.1.4) at a rate of 1 ml/min to 3 ml/min.

A.3.3 Sample elution

Weigh 5 g of betaine samples, accurate to 0,1 mg, in a 200 ml beaker (A.2.2), add 40 ml of acetonitrile solution (A.1.4), and stir until the sample is dissolved completely. Pass the sample solution quantitatively through the exchanger at a rate of 1 ml/min, add more acetonitrile solution (A.1.4) to eluate sample. Receive the eluate in a 100 ml volumetric flask (A.2.1) up to the mark for HPLC analysis.

Annex B
(informative)

Typical chromatogram

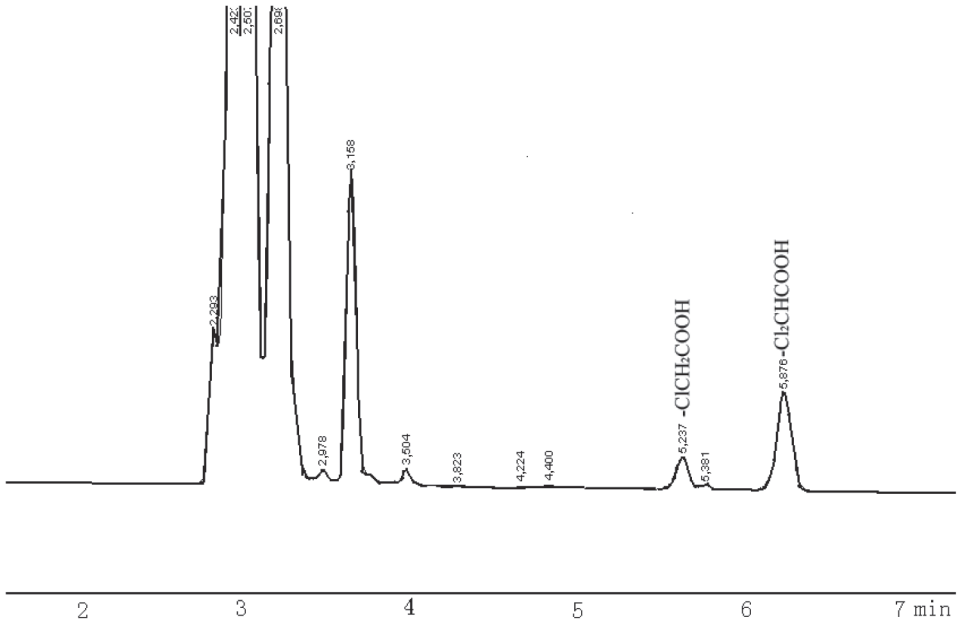

Figure B.1 — Typical HPLC chromatogram of monochloroacetic acid and dichloroacetic acid in betaine sample

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